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A STUDY OF SOMATIC CHROMOSOMES.

II. THE CHROMOSOMES IN EMBRYOS OF *Epilachna borealis* AND *Diabrotica vittata*.

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Epilachna borealis.

The oögenesis of *Epilachna* has not been described. Miss Stevens ('06) briefly described the spermatogenesis of this beetle. There are 18 chromosomes in the spermatogonia. One of these chromosomes, the y , is extremely small, its mate, the x , is larger. In each plate there are 2 chromosomes noticeably larger than the others. In the first maturation division the x -chromosome passes undivided to one pole of the spindle, while the y passes to the other pole. The spermatozoa, then, differ in that one half contain a small y , and the other half a larger x . Each contains one large macrochromosome (autosome).

Although the oögonial divisions and the maturation of the egg have not been worked out, the anticipated result of fertilization would be two sorts of embryos with respect to their chromosome content, one with two x 's, and one with an xy combination. This expectation is fulfilled in the divisions of the embryonic cells, as I shall show. The stages of development studied were: (1) Late cleavage, and (2) shortening of the elongate embryo. In all about 60 plates have been recorded.

Figs. 1-4 are from embryos having the xy combination in their chromosome complexes, these being the smallest chromosomes in the plate. Fig. 1 is a late cleavage division figure and shows the same characteristic elongation of the chromosomes as was seen in the case of *Anasa* at a similar stage of development (Hoy, '16). Figs. 2 and 3 are from cerebral neuroblasts, and are both from the same individual. Fig. 4 is from an ectoderm cell in the thoracic region. It will be seen that in all these figures there are two macrochromosomes comparable to those

found in the spermatogonia. Again, in all the figures the small y -chromosome is very conspicuous. The x -chromosome is here undoubtedly the next smallest in size, since this chromosome has no homologous mate. All the chromosomes show the typical elongate and more or less slender form characteristic of the Coleoptera. There do not seem to be any special peculiarities shown by the seven pairs of mesochromosomes, ranging between the macrochromosome pair and the xy pair, other than that they fall into a graded series according to size.

Plates from embryos containing no very small y -chromosome, but apparently having the small x -chromosome paired, are shown in Figs. 5-8. All these figures again show the large pair of macrochromosomes, and a series of seven paired mesochromosomes gradually diminishing in size. In none of the figures is there a small rounded y -chromosome, but in its place there is another the size of the x -chromosome.

It has, therefore, been possible to demonstrate that in *Epilachna*, where the male has an x - y pair in the spermatogonial complex, the embryos fall into two classes as regards their chromosome content, one class containing an xy pair, and the other 2 x 's.

RECORD OF COUNTS OF CHROMOSOMES IN *Epilachna*.

STAGE I.

Cleavage Mitoses. xy Type.

Embryo.	No. of Chromosomes.	No. of Counts.
1	18	1
2	18	8
3	18	2
4	18	1
5	18	2
6	18	2
		—
		Total . . . 16

Cleavage Mitoses. 2x Type.

Embryo.	No. of Chromosomes.	No. of Counts.
1	18	1
2	18	2
3	18	1
4	18	1
5	18	1
6	18	3
		—
		Total . . . 9

STAGE II.

Shortening of the Elongate Embryo, xy type.

Embryo.	No. of Chromosomes in Hypodermis.	No. of Counts.	No. of Chromosomes in Neuroblast.	No. of Counts.	No. of Chromosomes in Mesoderm.	No. of Counts.
1	18	1			18	1
2						
3			18	1		
4			18	1		
5	18	1				
6			18	3		
7	18	2				
8			18	2		
9			18	1		
10			18	2		
		Total 4		Total 10		Total 1

2x Type.

1	18	3				
2	18	1			18	1
3			18	2	18	1
4					18	2
5	18	1				
6					18	1
7			18	1		
8			18	1		
9			18	3		
10			18	1		
		Total 5		Total 8		Total 5

Diabrotica vittata.

Miss Stevens ('08) reports 21 chromosomes "of various sizes and shapes" in the spermatogonia of this species. Her figure shows one large macrochromosome pair. If arranged in a paired series graded in size, the unpaired *x*-chromosome would apparently be placed about ninth, followed by two pairs of smaller chromosomes. In the first maturation division the *x*-chromosome moves undivided to one pole of the spindle, giving them two classes of secondary spermatocytes. Of the resulting spermatozoa, one half contain an *x*-chromosome, and one half do not.

The second maturation of the egg was reported in a paper preliminary to this (Hoy, '14). Eleven chromosomes enter the female pronucleus. Fig. 12 shows the chromosomes of the first maturation division. There are eleven, each of them appearing

as a tetrad. They are clearly of different sizes, though the same size differences, such as appear in the spermatogonia, can not be observed.

Accordingly, two classes of embryos would be expected as the result of the fertilization, one containing 21, and the other 22 chromosomes in the embryonic cells. These differences have been observed and are shown in Figs. 9-11.

Figs. 9 and 10 are of the 21 chromosome type and are from the same embryo, corresponding in development to the stage of 3 of *Epilachna*. Fig. 11 is from an ectoderm cell of a late blastoderm stage of the 22 chromosome type. In all these figures one pair of chromosomes is much larger than any of the others. With the exception of the ninth chromosome in Figs. 9 and 10, all the other chromosomes form a paired series graded in size. In Fig. 11 there is no unpaired chromosome.

CONCLUSIONS.

1. The developing eggs and embryos of *Epilachna borealis* fall into two classes in respect to their chromatin content, one containing an *xy* combination, and the other an *xx* combination of sex chromosomes in all the cells of the body which have been examined, the former corresponding to the chromosomes of the spermatogonia, the latter probably to those of the oögonia. The same size relations are maintained in all the chromosome groups of one type.

2. Two types of chromosome groups are found in embryos of *Diabrotica vittata*, one with 21 chromosomes, corresponding to the spermatogonial chromosomes and one with 22, presumably homologous with those of the oögonia. In each of these types the chromosomes do not vary in number and form in different tissue cells which have been studied.

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'14 A Preliminary Account of the Chromosomes in the Embryos of *Anastrepha tristis* and *Diabrotica vittata*. BIOL. BULL., Vol. 27, No. 1.

'16 A Study of Somatic Chromosomes, I. BIOL. BULL., Vol. 31, No. 5.

Stevens, N. M.

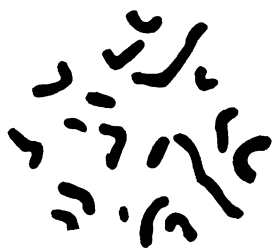
'06 Studies in Spermatogenesis. Pub. Carn. Inst. Wash., 36.

'08 The Chromosomes in *Diabrotica vittata*, *Diabrotica soror* and *Diabrotica 12-punctata*. Journ. Exp. Zool., Vol. 5, No. 4.

EXPLANATION OF PLATE I.

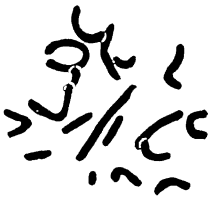
Epilachna xy Type.

- FIG. 1. Cleavage.
- FIG. 2. Neuroblast.
- FIG. 3. Neuroblast.
- FIG. 4. Ectoderm.



1

1 1 1 1 1 1 1 1 1 1
1 1 1 1 1 1 1 1 1 1



2

1 1 1 1 1 1 1 1 1 1
0 1 1 1 1 1 1 1 1 1



3

1 1 1 1 1 1 1 1 1 1
1 1 1 1 1 1 1 1 1 1



4

1 1 1 1 1 1 1 1 1 1
1 1 1 1 1 1 1 1 1 1

EXPLANATION OF PLATE II.

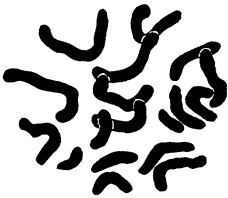
Epilachna xx Type.

FIG. 5. Cleavage.

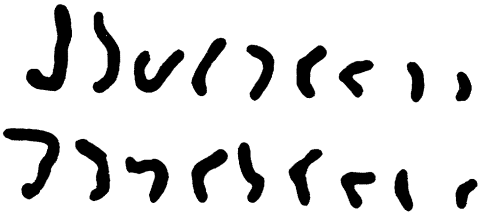
FIG. 6. Neuroblast.

FIG. 7. Neuroblast.

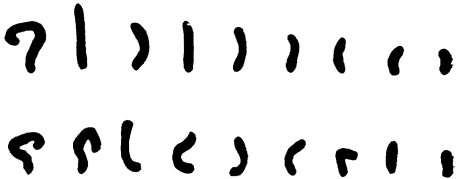
FIG. 8. Ectoderm.



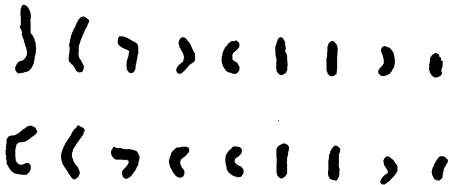
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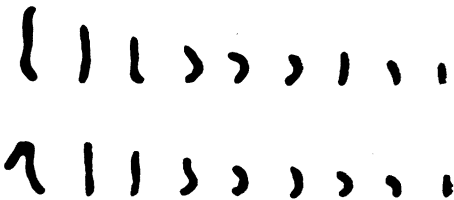
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7



8



EXPLANATION OF PLATE III.

Diabrotica.

- Fig. 9. Ectoderm, 21 chromosomes.
FIG. 10. Neuroblast, 21 chromosomes.
FIG. 11. Ectoderm, 22 chromosomes.
FIG. 12. Tetrads of first maturation division of the egg.



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9



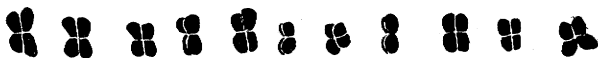
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10



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11



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